Fig. S1

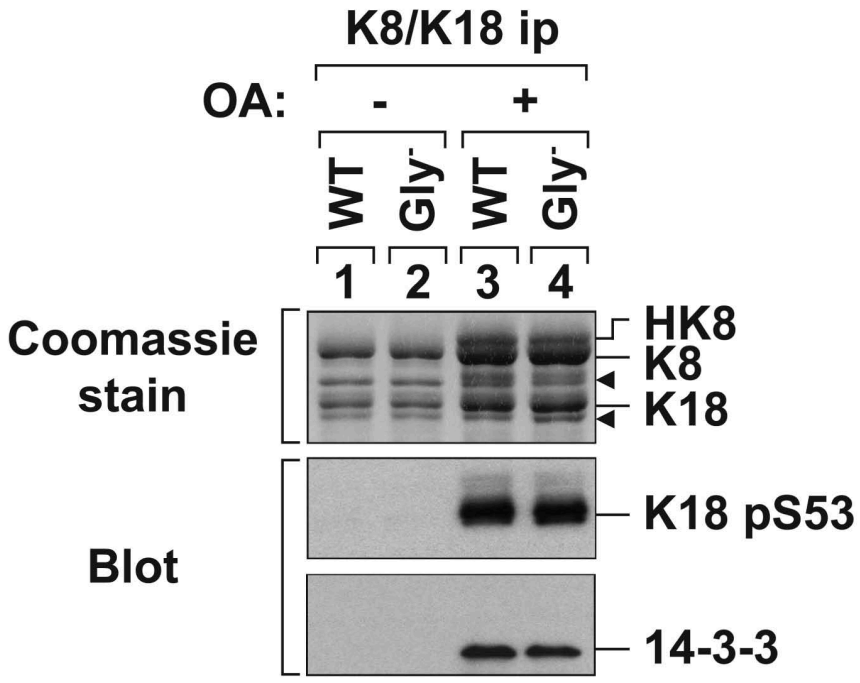
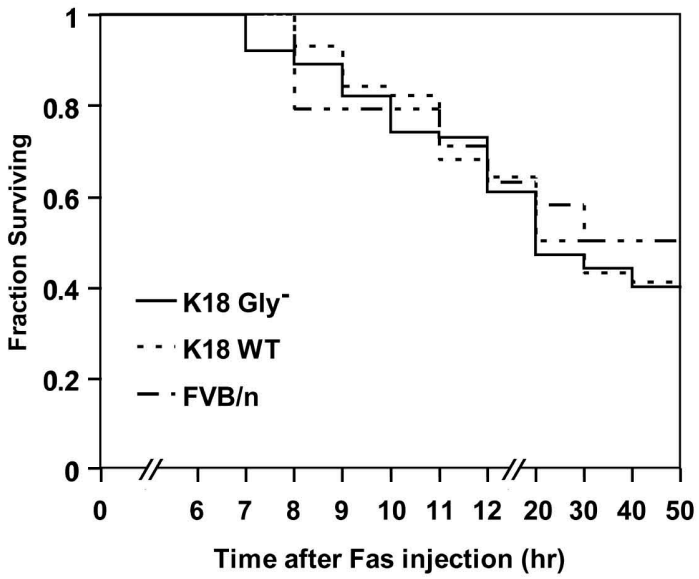


Figure S1. K18 Gly$^-$ (S30/31/49-to-A) does not affect K18 S34/S53 phosphorylation. BHK cells were co-transfected with K8-WT and K18-WT, or with K8-WT and K18-Gly$^-$. Transfected cells were cultured in the absence or presence of okadaic acid (1 μg/ml, 2 hr), a phosphatase inhibitor that leads to accumulation of phosphorylated proteins in cells. K8/K18 were immunoprecipitated and blotted with antibodies to K18 pS53 or 14-3-3 proteins (14-3-3 proteins bind to K18 pS34). HK8 represents K8 pS74 species (ie, a hyperphosphorylated form of K8). Arrowheads indicate degraded keratins. Note that K18 hyperphosphorylation is independent of mutating the K18 glycosylation sites.

Fig. S2

a



b

Mouse line	Dead mice	Total mice	Lethality %
FVB/n*	12	24	50
K18 WT	26	44	59
K18 Gly$^-$	37	62	60

* Nontransgenic mice

Figure S2. K18-Gly$^-$ mutation does not predispose to Fas-induced injury in transgenic mice. a, Age- and sex-matched mice from the three indicated genotypes (non-transgenic FVB/n, K18-WT and K18-Gly$^-$ mice) were given Fas ligand (antibody Jo2; Pharmingen) by intraperitoneal administration (0.15 mg/kg mouse body weight). Mice were then assessed hourly for the first 12 hr and then every 10 hr for 3 days. Most deaths were observed within the first 12 hr and no deaths were noted after 50 hr. **b,** Summary of mouse mortality described in panel (**a**).

Fig. S3

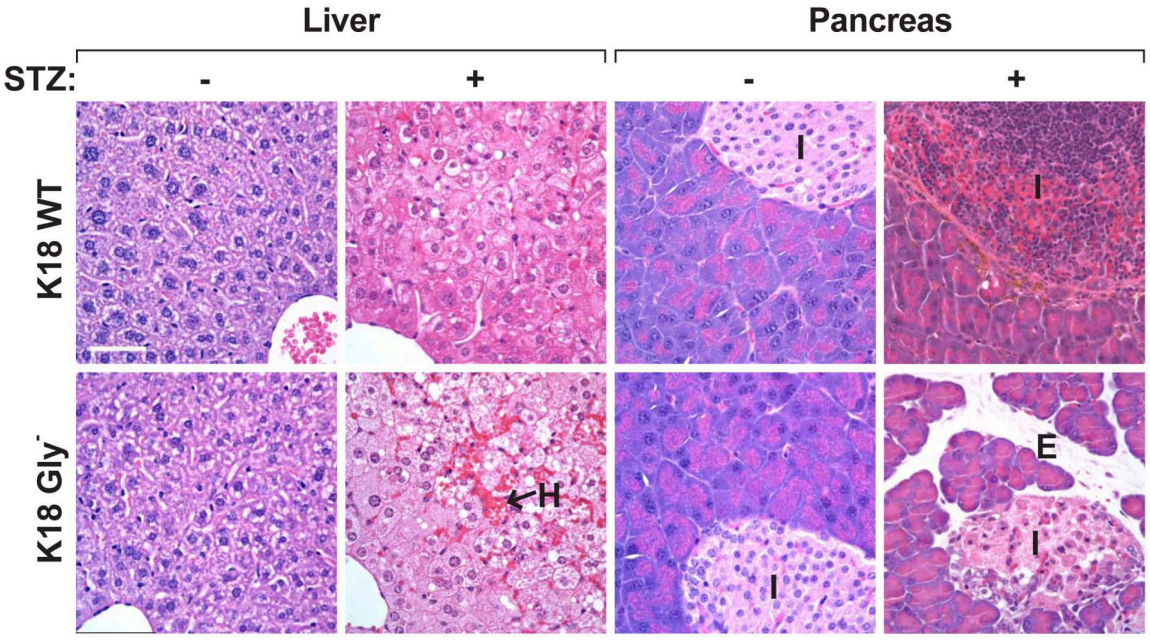


Figure S3. K18-Gly$^-$ mice increase susceptibility to STZ-induced tissue injury. Histopathology of mouse organs were compared before and 2 days after STZ injection. E, edema; H, hemorrhage; I, islet. Bar: 50 μm. This figure represents a higher magnification of what is shown in Fig2b.

Fig. S4

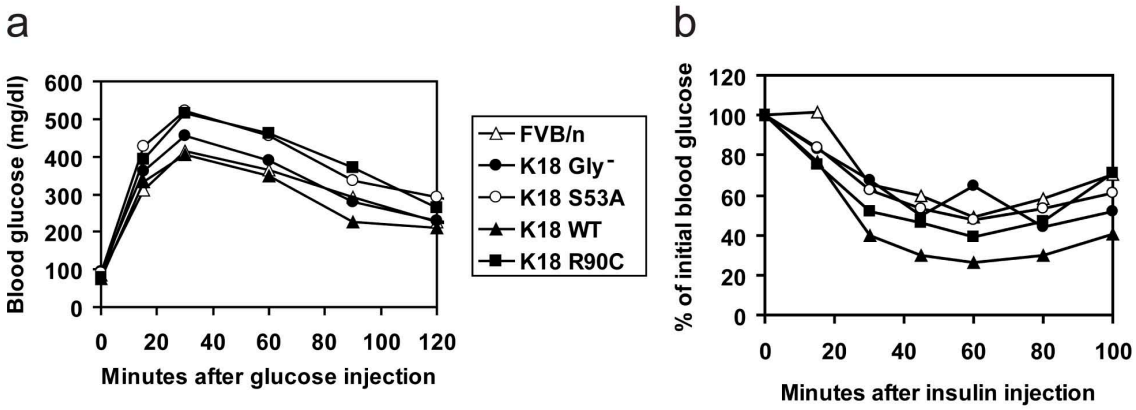


Figure S4. K18 mutations do not affect glucose and insulin tolerance in mice. a, Glucose tolerance test. Mice were fasted for 16 hr then blood was collected to measure fasting levels after which the mice were injected intraperitoneally with 2 g/kg body weight of glucose. Blood was then collected after 15, 30, 60, 90 and 120 min to measure glucose levels. **b,** Insulin tolerance test. Mice were injected with 1 U/kg body weight of insulin. Results are shown as mean of blood glucose concentration or % of initial blood glucose from at least 8 mice (age and sex matched) of each strain.

Fig. S5

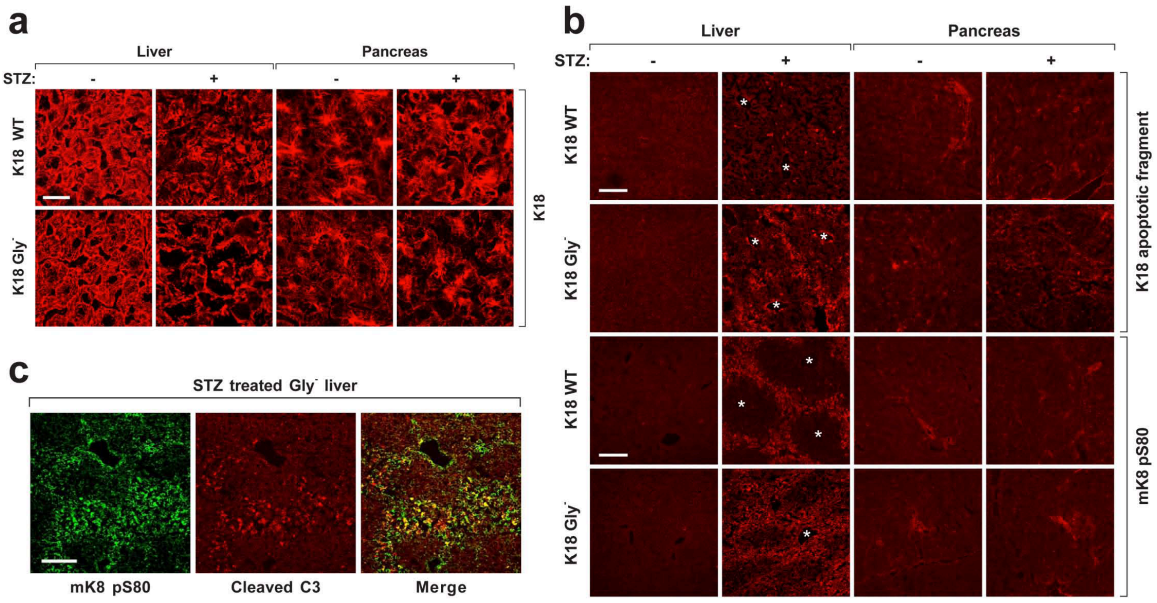


Figure S5. Immunostaining of liver and pancreas from STZ-treated mice. a, b, Isolated liver and pancreas, 2 days after STZ treatment, were analyzed by immunostaining using antibodies to K18 (**a**), K18 apoptotic fragment or to mK8 pS80 (**b**). Asterisks indicate blood vessels. Bars: 20 μm for panel (**a**), 200 μm for panel (**b**). **c,** Livers were obtained from STZ-treated K18-Gly$^-$ mice then double-stained with antibodies to the indicated antigens. Most apoptotic cells (red) colocalized with S80-phosphorylated cells (green) thereby providing the merged yellow color, but not all S80+ cells were apoptotic. This suggests that mK8 S80 phosphorylation occurs first and is then followed in some cells by apoptosis. The colocalization of cleaved C3 and mK8 pS80 in WT liver after STZ treatment was not detected due to very weak staining of cleaved C3 (not shown). Bar: 200 μm.

Fig. S6

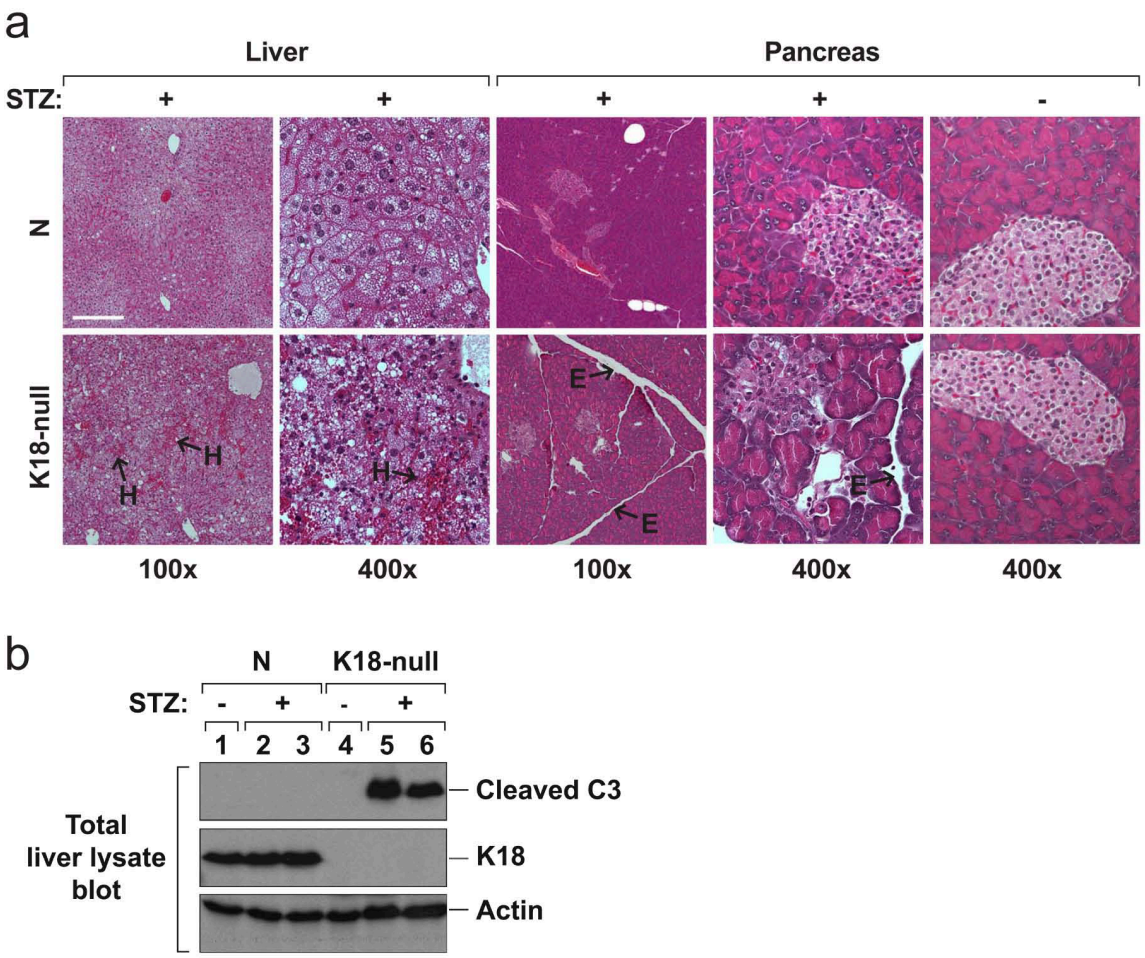


Figure S6. K18-null mice have increased susceptibility to STZ-mediated tissue injury. a, K18-null (backcrossed from its original mixed-strain background to an FVB/n background for 6 generations) and non-transgenic FVB/n mice were injected intraperitoneally with 200 mg/kg mouse body weight of STZ. After 2 days, liver and pancreas were isolated and stained with hematoxylin-eosin. Similar histological staining patterns were observed under basal conditions (not shown). However, after STZ treatment, the K18-null mice manifested more severe liver hemorrhage, pancreatic edema and islet cell necrosis as compared with the non-transgenic mice that had minimal liver hemorrhage and blood infiltration into the pancreatic islets. N, non-transgenic mice; H, hemorrhage; E, edema. Bar: 200 μm in 100x, 50 μm in 400x. **b,** Mice from the indicated genotypes were injected with STZ (200 mg/kg mouse body weight). After 2 days, total liver lysates were prepared from the STZ-treated or from control mice followed by blotting with antibodies to the indicated antigens. Each lane represents analysis of an independent mouse liver. The actin blot is included to demonstrate near-equal protein loading.